FURTHER OBSERVATIONS ON THE EMERGENCE OF DRAGONFLY-LARVÆ FROM THE EGG,

With Special Reference to the Problem of Respiration.

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(Five Text-figs.)

In March, 1914, I was fortunate enough to observe the process of hatching of some larvæ of *Anax papuensis* Burm., and was able to discover some interesting facts with regard to the pronymphal stage, the nature of the pronymphal sheath, the action of the peculiar organ which I have termed the cephalic heart, and the method by which gas first appears in the tracheal system of the emerging larva. These observations were published in a condensed form in a short paper,* read at the British Association Meeting in Sydney, on August 25th, 1914. Last year, when studying the problem of the physiology of respiration in these aquatic larvæ, I was struck with the importance of the question as to how the tracheal system first becomes filled with gas, and the possibility of a causal connection between the action of the cephalic heart and the expulsion of the blood from the tracheal tubes of the emerging larva. In a recent paper,† I have shown the importance of these questions as part of the larger question of the physiology of respiration in aquatic larvæ, and, in particular, their bearing on what I have termed the "Diffusion Theory" of Aquatic Respiration.

The opportunity of some further research on these difficult problems occurred on March 18th of this year, when I obtained, from the river at National Park, a piece of a large stem of the common Water-Milfoil (*Myriophyllum* sp.) containing more than sixty eggs of *Anax papuensis*. These eggs were laid in a regular spiral, down the stem, throughout a distance of about five inches in length, viz., that part of the stem from about an inch below water-level downwards. In the present paper, I propose to give an account of the important results obtained from some experiments carried on with these eggs, together with some further observations made upon the eggs of a Zygopterid dragonfly, obtained from the same locality in November, 1915. These latter were not determined with certainty, but there is little doubt that they belonged to *Austrolestes leda* Selys, a very common species around Sydney.

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* Report of the British Association for the Advancement of Science. Australian Meeting, Sydney, August, 1914, pp.424-425 (1915).

† These Proceedings, 1915, xl., Part 3, pp.422-437, Plate xlvii.

I desire to thank Dr. H. G. Chapman, Lecturer in Physiology in the University of Sydney, for some valuable suggestions and advice in connection with the experiments carried out on the eggs of *Anax*.

I propose to divide this paper into five sections, dealing with the several problems which present themselves for solution. These are:—

1. The exact origin of the first gas in the tracheæ of the emerging larva.
2. The composition of the gas in the tracheal system.
3. The nature and action of the cephalic heart.
4. The effect of abnormal conditions upon embryonic development.
5. Rectal respiration in newly-hatched Zygopterid larvæ.

Sections 1-4 represent the studies carried out on *Anax papuensis* (Anisoptera); Section 5 those on *Austrolestes leda* (Zygoptera).

Section 1.—*The Exact Origin of the first Gas in the Tracheæ of the Emerging Larva.*

In my former account of the emergence of the larva of *Anax papuensis* from the egg, I pointed out how difficult it was to make accurate observations, owing to the rapidity with which the hatching takes place. The pronymphal stage lasts only a few seconds, and the whole period of emergence occupies only half-a-minute or a little longer. During most of this time, convulsive struggles on the part of the emerging larva make direct observations very difficult. I was unable to see the actual beginning of the entry of the gas into the tracheal system of the larva. In the pronymph, the whole tracheal system is filled with a pale yellowish liquid, indistinguishable from the blood in the general body-cavity or hæmocœle, except that it contains no corpuscles. When, after a short but active struggle, the young larva is free from the pronymphal sheath, gas can be seen travelling down the main tracheal trunks from the anterior mid-gut region backwards. I was able to watch this gas gradually occupy the rectal region, where every tiny capillary in the gill-basket can be seen becoming filled with gas, in a very regular and beautiful manner.

Now we cannot be content with the imperfect observation that the gas comes into the tracheæ at some level anterior to the rectum. That is, indeed, an important point, since it removes the initial difficulty of understanding how rectal respiration could be carried on by diffusion of oxygen into the rectal capillaries, from the circum-ambient water. But it is not enough. What we need to find out, and what is precisely the most difficult fact to discover, owing to the rapid nature of the emergence, is *exactly* where, when, and how the first gas enters the tracheal system. The following method of solving the problem suggested itself to me, and proved more successful than I had dared to hope.

It is a well-known fact that dragonfly-eggs are always laid so as to avoid direct contact with the air, even for a few seconds. Such contact seems to be fatal, probably owing to the dessicating influence of air upon eggs constructed especially for submergence under water, or for existence in other moist conditions. In the case of *exophytic* eggs, *i.e.*, those laid *outside* the tissues of plants, a gelatinous envelope protects them from the air during the short time that elapses between their passage from the body of the female and their being dropped into water. In the case of *endophytic* eggs, *i.e.*, those laid *inside* the tissues of plants, the female bores with her ovipositor well into the cambium of the plant, and deposits her eggs in the moist tissues.

Thus it appears that considerable moisture is essential to the survival and development of the embryo. Consequently, the oxygen necessary for the metabolism of the growing embryo cannot be obtained *directly* from the air, but must be absorbed from the moist medium surrounding the egg. It appeared to me, therefore, that if I could lessen the necessary oxygen-supply in some manner, I might succeed in so weakening the embryo, that its emergence from the egg would take a long time to accomplish, instead of being completed vigorously in about half-a-minute. By this means, it would be possible to observe at leisure those processes which could only be imperfectly noted under normally active conditions.

Having brought the eggs of *Anax papuensis* safely home in their piece of stem of Water-Milfoil, I placed them in a fairly large Petri dish, with plenty of water. The stem was then broken up into small pieces, from which a number were selected for examination. From these pieces, *twenty-eight* eggs were extracted without any damage, and were cleared as far as possible from vegetable tissues clinging around them. Several eggs were inadvertently damaged, and were thrown away. *Eight* eggs were cut out from a hard part of the stem, and were left surrounded by small blocks of vegetable tissue. All the pieces of tissue not containing eggs were returned to the Petri dish, which also contained *twenty* eggs *in situ* in the remainder of the Milfoil stem.

The twenty-eight eggs free of vegetable tissues were then placed all together in a small crystal dish, two-thirds filled with tap-water, and covered with a glass slide. The eight eggs enclosed in small blocks of vegetable tissue were similarly placed in a second crystal dish. The Petri dish was also kept covered. The three dishes were only occasionally uncovered for purposes of examination. Fresh tap-water was only added sufficiently to make up for slight losses by evaporation.

If we now denote the twenty-eight eggs as Series A, the eight eggs as Series B, and the twenty normally placed eggs as Series C, we can easily state the object of the experiment. Firstly, Series A has not only been subjected to a serious limitation of possible oxygen-supply to be derived from the water, but it has been entirely deprived of any aid in this direction which the plant-tissues might offer. Secondly, Series B, though presumably partially restricted in its possible oxygen-supply from the water, has the advantage of still being able to obtain some gas from the surrounding plant tissues, assuming that such tissues play a part in the oxygenation of the egg. Thus, Series B becomes a *control* upon Series A. Thirdly, Series C, placed under normal conditions, plays the part of a control upon both Series A and B.

At the start, I examined the eggs to determine to what stage of embryonic development they had attained. I found that *all, except one*, had just completed the revolution of the embryo. This particular one was in Series A, and was kept under careful observation. It finally yielded very remarkable results. As the eggs of *Anax papuensis* normally take about three weeks to hatch, I concluded that my eggs had been laid on or about March 7th, and had still ten or more days to go before hatching. At first, I feared that development had proceeded too far already for my experiments to yield successful results. Fortunately, however, this was not the case.

On March 27th, *i.e.*, nine days after the experiments began, I examined a large number of the eggs with special reference to *pulse*. Those in Series C could not be well examined; but, as one or two eggs in the less opaque tissues showed embryos with the dorsal vessel pulsating fairly regularly, I concluded that all was well in this series, and turned my attention to Series B. Here, again, most of the eggs were not favourably placed for observation, and I was compelled to reduce the amount of vegetable tissue around several of the eggs to very small dimensions. I then found that the embryos appeared to be perfectly healthy, with a pulse of about 70 per minute. Turning next to Series A, I found that, in many of the embryos, I could not detect any visible signs of heart-beat. In a few, the heart was beating very slowly and intermittently—on an average, not more than four or five times per minute. It was thus clear that removal from the vegetable tissues had seriously affected the embryonic development of Series A. I at once introduced a considerable amount of fresh tap-water into the dish, fearing that otherwise I might not succeed in obtaining any larvæ at all from this Series.

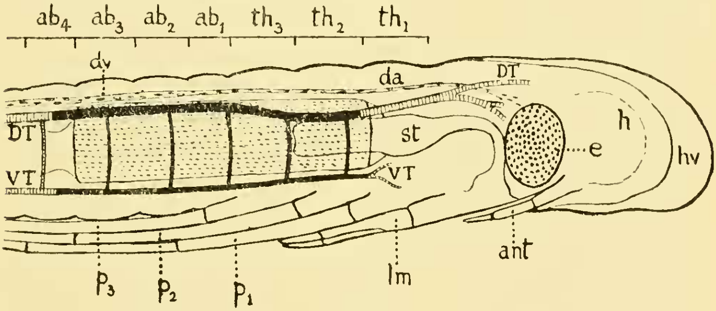
On March 28th, two larvæ had hatched out in Series C, and were normal and healthy. On March 29th, three more hatched out in Series C, and one in Series B. This last was transferred to the Petri dish, where it lived for three days. On March 30th, two more larvæ hatched from Series C, two from Series B, and two also from Series A. But these latter were both dead when I found them, though they could not have been hatched more than three hours before (*i.e.*, the time of my previous examination of the dish).

It was now necessary to spend every available moment watching Series A. For the four days, March 31st to April 3rd, I spent the whole of daylight and portion also of the nights in watching these eggs, only desisting for the shortest possible intervals for meals and rest. On March 31st, I selected in particular one embryo with a very slow but fairly regular pulse of about 20 to the minute, and watched it for four hours. I then desisted for half an hour's rest, and, on returning, found that this egg had hatched, and the larva was practically moribund. The next day, I watched the eggs for fourteen hours, selecting four especially that showed a fairly regular pulse. But I met with no luck, as not a single egg hatched on that day.

On April 2nd, I got up very early, and was rewarded by finding one of the four eggs above mentioned *just in process of hatching*. I was just in time to see the pronymph pass out from the egg-shell. The cephalic heart was acting very sluggishly; in fact, I had barely time to locate it before it subsided. The pronymph failed to pass completely out of the egg-shell, and remained with the last three or four abdominal segments inside it. The pulse of the dorsal vessel was 52 per minute. The larva made repeated attempts to remove the pronymphal sheath, by moving its head up and down, and also by forcing its midgut forwards. The œsophageal valve became deeply sunk into the midgut, where it was worked strongly up and down. The midgut was gradually forced further and further forward, until it came to occupy a considerable part of the thorax, as well as the first three or four abdominal segments. At the end of an hour, the pulse had dropped to 48; after three hours, it had fallen to 44. Half-an-hour later, it was down to 40. It then appeared that the pronymph was completely exhausted. I therefore removed it to a watch-glass, and fixed it immediately in hot water at 95°C.

It will be seen that this pronymph existed for about three and a half hours, and never succeeded in bursting open its sheath. During all this time, my attention was principally concentrated upon the tracheal system. As both egg-shell and pronymph lay upon their sides, and as the struggles of the latter to free itself were not sufficiently violent to displace it from this favourable position, I had a unique opportunity of watching for the appearance of gas. When the pronymph first emerged from the egg-shell, the whole tracheal system was clearly filled with a pale yellowish fluid (which could scarcely be anything else than the clear liquid part of the blood) without any corpuscles. A similarly coloured liquid could be seen passing along the dorsal vessel, accompanied by a small number of oat-shaped corpuscles. The whole head-cavity, and all the thorax and abdomen, except the midgut, also appeared to be full of the same coloured liquid. The midgut showed up as a darker cylindrical barrel or plug, evidently enclosing the remains of the yolk. The dorsal tracheal trunks could be easily made out, and their prolongations into the head could be followed as far as the eyes. The rectum could be distinguished, but no details of the tracheation of the gills could be made out. The ventral trunks could be picked up only with difficulty, as they were so much smaller than the dorsals. At no time was I able to see the visceral trunks, owing to the dark mass of the midgut being placed so close to them.

For three hours, I watched the tracheal system of this pronymph *without detecting any sign of gas in it.* A few minutes later, I thought that the dorsal trunks were beginning to become a little more distinct. Watching very intently, I now perceived that their outline was becoming definitely harder, and that the yellow liquid was most certainly being slowly replaced by gas. In ten minutes, the outline of the dorsal tracheal trunks in the meso- and metathorax, and in the first four segments of the abdomen, had become so hardened up that it was quite evident that this portion of the tracheal system was now filled with gas. Turning my attention to the ventral trunks, I found that these also were just in process of being filled with gas. At the end of a quarter of an hour, the parts of the tracheal system filled with gas were the dorsal and ventral trunks between the two ends of the midgut (in this case, in segments 1-4 of abdomen and 2-3 of thorax), and the five branches connecting them. This is shown in Text-fig.1, where the parts filled with gas are represented in black. No gas could be seen in the visceral tracheæ, which remained, as before, practically invisible.



Text-fig.1.—Anterior portion of pronymph of *Anax papuensis* Burm., to show the entry of gas into the tracheal system. Lateral view. Diagrammatic. The part of the tracheal system into which the gas entered is represented in black; the midgut is lightly shaded; (×55): *ab_1-ab_4*, abdominal segments; *ant*, antenna; *da*, dorsal aorta; DT, right dorsal tracheal trunk; *dv*, dorsal vessel or heart; *e*, compound eye; *h*, head of larva; *hv*, head-vesicle; *lm*, labial mask; *p_1-p_3*, legs; *st*, stomodæum or foregut; *th_1-th_3*, thoracic segments; VT, right ventral tracheal trunk.

During the next five minutes, the gas in the dorsal tracheæ extended gradually as far back as the sixth abdominal segment, and slightly forward into the prothorax. It never reached the branchial basket and the head, nor did any get into the visceral trunks viâ their connection with the dorsals, which, in this pronymph, lay in the anterior portion of segment 7. As no further changes took place, and the pronymph was evidently utterly exhausted, it was killed as described above.

It seems to me very important to emphasise *the very gradual and even appearance* of the gas in the tracheæ. *No separate bubbles of gas were to be seen;* in other words, *the gas did not come in at separate points*, but passed slowly into the tracheæ over a large area represented by two thoracic and four abdominal segments (roughly defined by the limits of the midgut). The process, as I watched it, can only be compared with the *slow development* of a retarded photographic plate. At first, it was impossible to say whether any change had taken place or not, and it was only after several minutes that one could realise the steady growth in distinctness of outline in the tracheæ into which the gas was entering.

By great good fortune, a second egg hatched out about an hour and a half after the one which I was observing. This egg had been placed close alongside the latter, as it appeared to me to be on the verge of hatching. I had forgotten its existence, until, quite suddenly, the head-capsule of the emerging pronymph appeared in the field of vision, and pushed gently against the pronymph which I was watching. This pronymph was even weaker than the one I had been watching, and only succeeded in getting about half-way out of the egg-shell. I could not locate the cephalic heart at all, and it must have ceased pulsating before the pronymph came into my field of view. As this egg-shell and pronymph were also lying on their sides, I took a fine needle, and gently pushed them so that they came to lie exactly alongside the one I was observing. Thus I had the midgut regions of two pronymphs under observation at the same time. The most important result of this was, that when the changes began in the tracheal system of the first pronymph, the tracheal system of the second, remaining filled with liquid, served as a most valuable means of gauging the extent of the change. But for this, I doubt if I should have noticed the gradual beginning of the change at all.

The behaviour of the second pronymph was not exactly the same as that of the first. After remaining weak and inert for about two hours (by which time the first pronymph had been just disposed of), I found that it had become much more vigorous, making many attempts to burst the pronymphal sheath, all of which, unfortunately, were failures. A count of the pulse, during the most active of these attempts, showed 70 beats to the minute. A few minutes later, I took a fine brush, and coaxed the pronymph out from its egg-shell. It at once began to bend up and down vigorously, and made frantic efforts to break its sheath. Soon after, I noticed the pulse quicken greatly, and a count gave 120 beats to the minute. On searching for the cause of this, I found that the sheath had burst dorsally over the sixth abdominal segment, and that the larval intestine was projecting through the slit. This, of course, meant a speedy death to the larva. This second pronymph, therefore, lived just over two hours, and no gas appeared in its tracheal system during that period.

Watch was continued throughout April 3rd. On that day, two more pronymphs emerged, both, unfortunately, after dark, and at a time when I was only able to pay occasional visits to the microscope. Completely tired out with the four days' watching, I was in no mood to continue the watch far into the night, and contented myself with noting that the general course of events, in the cases of these two, was very similar to that in the first pronymph. The tracheal system showed no signs of gas until about three hours after hatching; and the gas, when present, did not extend beyond the limits stated in the first case. Both these nymphs were found dead in the morning, having failed to burst their sheaths.

Before describing the remaining experiments made upon these eggs, it would be best to give in tabular form the full record of emergences for the Series A, B, and C, in order that references to the table may make these experiments clearer.

Table showing hatchings of Eggs of *Anax papuensis*, March 28th-April 11th, 1916.

Date.	Series A.	Series B (control upon A).	Series C (control upon A and B).
	28 eggs in tap-water without vegetable tissues.	8 eggs in tap-water with small pieces of vegetable tissues *in situ* around them.	20 eggs in original stem of Water-Milfoil, with plenty of water in Petri dish.
March—			
Tues. 28th	—	—	2L
Wed. 29th	—	1L	3L
Thurs. 30th	2L	2L	2L
Fri. 31st	1L	2L	3L
April—			
Sat. 1st	—	1L	4L
Sun. 2nd	2P	—	3L
Mon. 3rd	2P	—	—
Tues. 4th	—	—	2L
Wed. 5th	1P	—	1L
Thurs. 6th	—	—	—
Fri. 7th	1P*	1L	—
Sat. 8th	1L	—	—
Sun. 9th	—	1L	—
Mon. 10th	2L	—	—
Left over on April 11th	16 dead embryos	nil	nil
Total hatched	6P+6L	8L	20L

L denotes that the emerging embryo reached the full larval stage, P that it did not succeed in getting beyond the pronymphal stage. None of the larvæ obtained from Series A lived more than two or three hours. The pronymph marked * was from the unreversed embryo, and came out of the egg-shell *backwards*.

Section 2.—*The Composition of the Gas in the Tracheal System.*

The question next arose—What could be the composition of the gas which I had seen enter the tracheal system from the body of the pronymph? It seemed to me, as soon as I observed the phenomenon, that it must be one of two things, viz., (*a*) carbonic acid gas, or (*b*) oxygen. The former would naturally appear to be the most likely solution, since CO_2 is the only gas formed in large quantities within the body of a living animal. On the other hand, one naturally recoiled at the idea that such a gas should completely fill the *respiratory system* of the larva. The second solution, that it might be oxygen, would require the existence of some oxygenating substance in the blood, which would act on the CO_2 produced by metabolism in the developing embryo, and release oxygen therefrom, in some manner analogous to that of chlorophyll in the leaves of plants. But, though the blood of Odonata is always of a yellowish or greenish colour, we have it, on the high authority of Cuénot,* that the pigments *hæmoxanthine*, *hæmochlorine*, and *hæmoprasine*, which are the only substances that might conceivably perform such a function, are quite absent from the blood of these insects.

It seemed necessary, in any case, to obtain some definite result by experiment, and not to rely upon arguments alone, however conclusive they might appear. Unfortunately, by the evening of April 3rd, as the table already given will show, most of my eggs had already hatched, and I could not expect to obtain many more larvæ. I was forced, therefore, to limit my experiments, both in variety and in the number of larvæ on which they could be performed. I decided to employ only two test-solutions, whose combined results should indicate, with considerable certainty, the composition of the gas in the tracheæ. These were

(*a*) a 10% solution of caustic potash.
(*b*) a 4% solution of Azol.†

As a stand-by, I also prepared, (*c*) a 4% solution of caustic soda, in case solution (*a*) should prove too strong.

Control Experiments:—As a control upon the experiments, two live larvæ were taken from the Petri dish (Series C), and killed by being placed in a tube of water, in which a single drop of chloroform had been well shaken up. They were then removed to tap-water in a crystal dish, and the times taken for the tracheal system to collapse were noted.

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* Etudes sur le sang et les glandes lymphatiques dans la série animale, 2^{me} partie. Invertébrés. Arch. Zool. expér. 2^{me} série. ix., 1891.

† Azol, the proprietary name for an excellent, one-solution, photographic developer, which acts as a very strong reducing agent. The correct strength for use on plates is 1 part in 24 of water, or roughly 4%.

Result:—The rectal capillaries collapsed in from 2-3 hours, the main tracheal system (whole length of dorsal trunks) in from 5-6 hours.

Experiment 1.—Two larvæ, aged from one to two days old (strongly pigmented), were placed alive in 10% solution of KOH. The solution was regularly sucked into the rectum and expelled again, causing considerable irritation to the larvæ, both of which died within an hour. A close examination showed that the rectum became swollen and somewhat opaque. The rectal capillaries remained quite unaffected, except where the gill-tissues became very badly damaged. The main tracheal system was not affected in any way.

Conclusion.—No appreciable CO_2 present in the tracheal system of larvæ 1-2 days old.

Experiment 2.—Two larvæ, which emerged from Series C on April 4th, were taken before they were two hours old, and placed alive in 10% solution of KOH. Both died within half-an-hour, after having repeatedly sucked the solution into the rectum and expelled it again. In three-quarters of an hour, the dorsal trunks began to collapse from the region of the rectum forwards, but the rectal capillaries remained unaltered. At one hour, the dorsal trunks were very badly collapsed. At two hours, all the tracheæ in the abdomen had collapsed, except the rectal capillaries, which remained only partially collapsed. At three hours, the whole tracheal system had collapsed, including the parts in the head and thorax, but there still remained a number of the rectal capillaries only partially collapsed. At four hours, all the capillaries had collapsed (*cf.* Control Experiment).

Conclusion.—The gas in the tracheal system of larvæ 1-2 *hours* old is CO_2, except in the rectal capillaries, which are, at any rate, partially filled with some gas not extracted by KOH.

Experiment 3.—Two larvæ were taken from Series B and C respectively, the latter between two and three days old (strongly pigmented), the former between six and twelve hours old (just beginning to become pigmented), were placed alive in 4% solution of Azol. The larvæ lived for two or three hours, and appeared to suffer little inconvenience or irritation, though the liquid was repeatedly sucked into the rectum. In both larvæ, the main tracheal system remained intact *for twelve hours* (a very striking result, *cf.* Control Experiment). In the younger larva, the rectal capillaries remained absolutely intact at the end of twelve hours (again, *cf.* Control Experiment). In the older larva, the rectal capillaries collapsed partially at the end of three hours.

Conclusion.—The action of the Azol is clearly resisted by chitin, and hence, no safe conclusions can be drawn as to what gas was present in the main tracheal system. In the rectal capillaries, there was little or no oxygen present in the case of the younger larva, but enough oxygen was present in the case of the older larva to affect the stability of these tubes when it was withdrawn by the Azol.

Experiment 4.—A moribund larva, taken from Series A, soon after hatching, was placed in a solution of 4% NaOH. This larva remained inert, without sucking the solution into its rectum, and died within an hour. *Result*, same as stated for the larvæ in the Control Experiment.

Conclusion.—No guide as to composition of gas. Either the solution was too weak to penetrate the chitin, or else the fact that the larva was moribund, and did not draw the solution into its rectum, prevented contact between the solution and the most permeable part of the animal (*i.e.*, the thin, chitinous cuticle of the rectal gills).

Besides the larvæ used in the above experiments, a single larva emerged from Series B on April 9th, at a time when I was unable to make use of it, and two moribund larvæ emerged from Series A on April 10th, dying before I could make use of them. No more larvæ emerged, and no less than *sixteen* dead embryos remained inside eggs of Series A until April 20th, when they were thrown away.

The results of my experiments, few though they were in number, appear to point definitely in the following direction:—While there is no appreciable quantity of CO_2 in the tracheal system of larvæ more than a day old (Expt. 1), yet, in larvæ only an hour or two old, the whole system is filled with CO_2, except the rectal capillaries, where there is a mixture of gases (Expt. 2). Also, though the Azol solution appeared to be unsuitable for penetration of chitinous membranes, yet the fact that it extracted some of the gas from the rectal capillaries of a 2-3 days old larva, and failed to do so in the case of a 6-12 hours old larva, suggests that the proportion of O in the gills of the former was considerably greater than that in the gills of the latter (Expt. 3). We are thus led, on all three counts, to the definite conclusion that, (i.) *the original gas which fills the tracheal system of the larva during the process of hatching is* CO_2, but that, (ii.) *the establishment of regular rectal respiration sets up a process of diffusion between the rectal capillaries and the water in the rectum, whereby the* CO_2 *in the tracheal system is gradually withdrawn, and replaced by air, or a mixture of nitrogen and oxygen not differing much from the ordinary composition of air.*

It is easy to show that these results are in agreement with the physiological aspects of the problem. Firstly, the exertions of the larva to burst open both the egg and the pronymphal sheath involve a sudden and rapid increase of metabolic activity. Thus, just at the period when large quantities of CO_2 are being liberated by the activities of the larva, gas replaces the liquid in the tracheæ. If this gas be CO_2, as our experiments suggest, we have solved at once the problem of what the larva does with the large amount of CO_2 liberated by its new exertions, and why it is that the gas comes into the tracheal system in the manner it does. Secondly, if we grant that this gas is CO_2, the gradual change to air, or a mixture of gases not differing appreciably from air, is not only to be expected as a result of rectal respiration, but must inevitably take place, by the ordinary laws of diffusion. For, in the tracheal system of the newly-hatched larva, the pressure of CO_2 will be 760 mm., or atmospheric pressure. But in the aërated water of the rectum, the partial pressure of O is approximately 150 mm., that of N approximately 610 mm., and that of CO_2 less than 1 mm. It follows that the

CO_2 in the tracheæ must *eventually* be replaced *almost completely* by a mixture of O and N closely resembling air. (As the larva must be continually using up the O, it is not necessary to assume that the *total* amount of gas in the tracheæ at any given moment, if analysed, must have the *exact* composition of air). The rate at which the exchange takes place will be governed by the morphological conditions in the rectum. As the expanse of the rectal chamber is limited, only a certain amount of aërated water can be drawn in at one time. Hence, if this water bathes any given length of cylindrical tracheal tubing, it is clear that, the smaller the bore of the tube, the quicker will the exchange of gases take place. The rate of exchange of gas may be roughly taken as inversely proportional to the area of the cross-section of the tube, *i.e.*, to the square of the diameter of the tube. Also, any increase in the length of tube exposed to the water will facilitate the rate of exchange. Thus *the ideal conditions for a rapid exchange are fulfilled in the case of a large number of exceedingly fine tubes having a great portion of their length bathed by the water.* This is exactly the morphological condition developed in the rectal gills of Anisopterid larvæ.*

Section 3.—*The Nature and Action of the Cephalic Heart.*

In my previous study upon the larva of *Anax papuensis* (*l.c.*), I described the action of the peculiar pumping-organ of the head, and suggested for it the name of the *cephalic heart.* Owing to the rapidity of the act of hatching, and the fact that this organ

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* *Note.*—In a letter received from Mr. G. L. Purser, B.A., of Trinity College, Cambridge, in which he discusses my paper on the Physiology of the Rectal Gills, he remarks on the peculiar problem of the first appearance of gas in the tracheæ:—"I don't see why the gas should appear just where it does. My idea is this: the gas appears first in the thickest part of the embryo; it appears just when the larva is making a series of strong muscular efforts for the first time. Why, therefore, should it not be CO_2? The gas appears where CO_2 will be most concentrated; it appears *when* it is most concentrated." This letter was received only a few days after my experiments were completed, and was, therefore, written some time before them. I desire, therefore, to give Mr. Purser due credit for the first conception of the idea stated in his letter.—R.J.T.

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is only in action for about half-a-minute, it is most difficult to determine its exact nature and position. We know that it is situated above and somewhat posterior to the mouth, and below the dorsal aorta; that at first it is very small, that it increases rapidly in size and in the force of its pulsations; and then, after performing about twenty-five regular double-beats in half-a-minute, subsides very quickly. In appearance, it is two-chambered, but whether there are actually two pulsating chambers, or a single one constricted at some point by the tentorial structures of the head, I had not been able to determine. With Balfour Browne,* I agree that this organ pumps a pale yellowish liquid, which can be none other than the liquid part of the blood. I did not observe any corpuscles passing through the organ. Balfour-Browne states that the blood is pumped upwards and backwards. This appears to me also to be correct as regards the first few pulsations of the organ; after that, I confess that I could not follow a definite course of the blood, and I must hold to the opinion that most of the blood pumped into the anterior chamber (or *auricle*, as I previously termed it) was again pumped out into the posterior chamber (or *ventricle*), and served to cause the immense distension of that chamber which takes place just before the larva bursts its pronymphal sheath. What the exact nature of the organ is, and whence and whither the blood was actually pumped, I had not been able to determine.

In entering upon the experiments already detailed in Section 1 of this paper, I had strong hopes that the general retardation of the process of hatching might be accompanied by a corresponding retardation in the action of the cephalic heart, and that thus I might be able to examine this organ more minutely. It will be seen already, however, that these hopes were frustrated, since the cephalic heart barely came into action before it subsided again, at the birth of the pronymph, and failed altogether to come into action again during the whole of the pronymphal stage, lasting over three hours. Indeed, it is very probable that the failure of the larva to emerge from the pronymphal sheath was correlated with its failure to bring this strong pumping apparatus into action, to aid in the required distension of the head.

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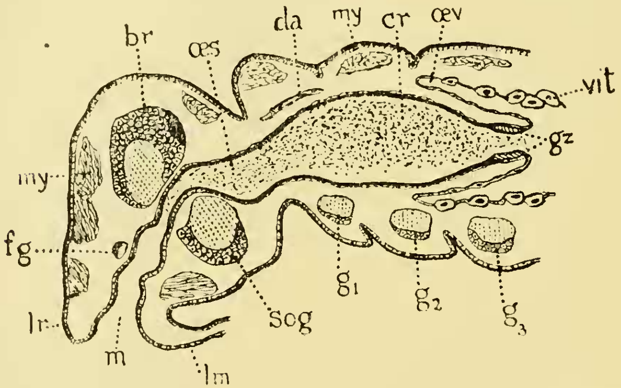
* "The Life-History of the Agrionid Dragonfly." Proc. Zool. Soc. London, 1900, pp.253-285.

My one remaining hope of discovering something about this important organ lay in a study of sections of a pronymph. As already stated, the pronymph studied on p.394 was removed in a moribund condition, after it had lived for three and a half hours, and was fixed in hot water. Though it was almost dead when the hot water was poured upon it, the sudden contact with this liquid caused it to burst the pronymphal sheath, and flow completely out of it. Thus the animal that I had fixed was, in a sense, the true larva, but *with the form of the pronymph*; for its eyes and head did not expand to the true larval form, its labium and legs remained directed backwards as in the embryo, and its abdomen was still of the slender form seen in the pronymph. This little animal was carefully double-embedded in celloidin and paraffin, and cut into sagittal sections of 7μ thickness. One of these, a little to the right of the median sagittal plane, is shown in Text-fig.2.

In order to understand this section, I have also shown, in Text-fig.3, a drawing which I made, by means of the camera lucida, of the anterior portion of the same pronymph, at about two hours old. It must be remembered that the cephalic heart had ceased to beat almost directly after the pronymph had hatched. Thus we cannot see the shape and position of that organ in the drawing; and if we want to search for it in the sections, we must bear in mind that it had either collapsed, or assumed some stable form prior to the period of fixation. Text-fig.3 shows us the exact course of the blood-circulation in the head-region during the pronymphal stage, and also the peculiar position of the midgut, pushed forward into the thorax, with the œsophageal valve and gizzard sunk deeply into it, and the crop projecting in front of it, just below the end of the dorsal aorta.

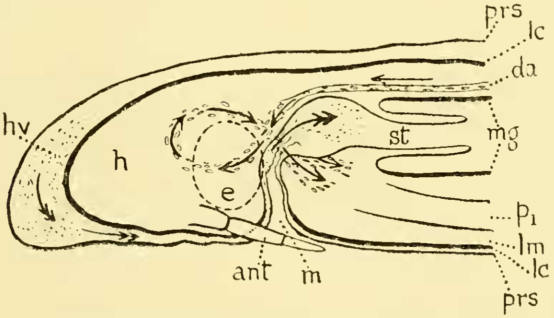
Now, if we study the series of sections, we at once notice the very remarkable fact that, at the time of fixation, *the anterior* *œsophageal region is strongly distended with blood, in which, however, no corpuscles are visible.* A search through the whole series of sections likewise fails to show the presence of any special organ which might be interpreted as the collapsed cephalic heart. There is nothing to be seen except the usual organs of the head and thorax present in any insect-larva. If we combine these two facts together, the conclusion is very strong that the cephalic heart is really only *a special temporary development in the œsophagus*, and that, therefore, the pumping of the liquid blood must take place *through the mouth.*



Text-fig. 2.—Nearly median, sagittal section through pronymph of *Anax papuensis* Burm., with the pronymphal sheath removed. To show mass of blood-coagulum, without corpuscles, distending the œsophagus and crop. (× 100): *br*, brain; *cr*, crop; *da*, dorsal aorta; *fg*, frontal ganglion; g_1-g_3, thoracic ganglia; *gz*, gizzard; *lm*, labial mask (cut off); *lr*, labrum; *m*, mouth; *my*, muscle; *œs*, œsophagus; *œv*, œsophageal valve (junction of fore- and midgut); *sog*, subœsophageal ganglion; *vit*, vitellophags in wall of midgut.

Let us now see how this conclusion agrees with the observed facts of the hatching of the egg. Before the pronymph emerges, there is a large "*head vesicle*" fitting closely under the pedicel of the egg. When the cephalic heart comes into action, the liquid in this vesicle (which must obviously be a space between the pronymphal sheath and the true larval head) is withdrawn, the head of the larva swells up greatly, and, by this means, the pedicel of the egg is pushed aside, and the pronymph emerges. After a short period of time (from a few seconds to half-a-minute at the most in *Anax*), the activity of the cephalic heart increases rapidly, the head again swells up, but this time to almost twice its previous size, and the larva escapes through the dorsal splitting of the pronymphal sheath.

Now there is no possible connection between the "head-vesicle" and the interior of the embryo, except viâ the larval mouth. If the cephalic heart be merely a temporary formation in the œsophagus, acting somewhat in the manner of a sucking-pharynx, it would be able to exhaust all the liquid in the "head-vesicle," by sucking it into the lumen of the œsophagus. Text-fig.3 shows, in a diagrammatic manner, how this would be done. The accumulation of all this volume of liquid in the anterior part of the œsophagus, together with the extra pressure caused by the



Text-fig.3.—Head and portion of thorax of pronymph of *Anax papuensis* Burm., to show, (*a*) course of blood-corpuscles in the head-region (single arrows), and (*b*) suggested course of liquid blood (lymph) drawn into œsophagus by action of cephalic heart (double arrows). Diagrammatic. (×60): *ant*, antenna; *da*, dorsal aorta; *e*, eye (dotted outline); *h*, larval head; *hv*, head-vesicle; *lc*, larval cuticle; *lm*, labial mask; *m*, mouth; *mg*, midgut; p_1, foreleg; *prs*, pronymphal sheath; *st*, stomodæum or foregut.

forward movement of the midgut, would be quite sufficient to cause the splitting of the pronymphal sheath, as well as the lifting-up of the egg-pedicel.

As regards the two-chambered structure of the cephalic heart, a reference to the section in Text-fig.2 shows the exact position where the tentorium would cause a definite constriction in the œsophagus, if that organ were to distend itself in any manner. The passage through this tentorial foramen, below the brain(*br*) and above the subœsophageal ganglion (*sog*) is small, and the tentorial bars are hard and rigid. Moreover, the œsophagus, both at this point and throughout its entire length, is capable of very great expansion, as may be seen in any transverse section of that organ, both by reason of the longitudinal pleating or infolding of its epithelium, and the strength of its muscular tunic. It seems reasonable to suppose that the division into two chambers is caused by the tentorial compression. The notes and drawings that I made for my previous paper show the point of constriction placed somewhat further back than the position of the tentorium. I think, however, that too much weight must not be placed upon this fact, as it was manifestly impossible to make accurate drawings of all the different parts of the larva, during the single half-minute of vigorous struggle for emergence. If, however, future observations should confirm my original drawings,* then there is reason to suspect the existence of a special sphincter muscle at some definite position on the œsophagus. Such a structure would have to be searched for in transverse sections.

Section 4.—*The effect of abnormal conditions upon embryonic development.*

We can now turn to some problems of a more general nature arising from the experiments described in Section 1.

If we examine the table of results given on p.398, we see that Series B and C gave identical results, *viz.*, in both cases all the eggs hatched, and yielded healthy larvæ. We must conclude, therefore, that the restriction of the amount of water allowed to Series B, and the covering-over of the crystal dish (except on rare occasions for a few moments) by a glass slide, to prevent free aëration of the water, had no appreciable effect upon the course of development.

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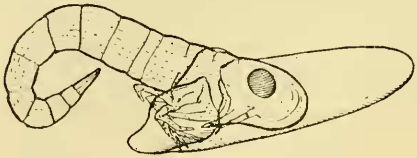
* These will be found in Fig.24 of my book, "The Biology of Dragonflies," shortly to be published by the Cambridge University Press.

The only difference between Series A and Series B was *the removal of the vegetable tissues* from around the eggs of the former series. The results, as we have seen, were disastrous. Sixteen embryos died, six reached the pronymphal stage, and six just got as far as the larval stage, but were too weak to go on living. We can only conclude that *the vegetable tissues play an important part in the oxygenation of the embryo.*

Let us now consider the peculiar case of the pronymph in Series A, which emerged on April 7th (marked 1P* in the Table). As already stated, this was the only embryo in Series A, which, when first subjected to the conditions of the experiment, had not undergone the process of reversion. The fact that it was already behind the rest of the eggs in development might suggest some inherent weakness in this particular individual; but there is no reason to suppose that it would not have gone on developing normally, if left to itself. That it was not parasitised is clear from the fact that it finally hatched out. Also, since the eggs were all laid together in a regular spiral, on the single piece of stem, we have no reason to believe that it was not one of the same batch of eggs as all the rest. The fact that, in Series C, under normal conditions, the eggs continued to hatch over a period of nine days, shows us that considerable variations in the length of embryonic existence are to be expected. This egg merely happened to be the most backward of the whole set. The effect of the new conditions upon it was most marked, because it had developed less far under normal conditions. It was, however, a very unexpected and extraordinary thing, to my mind, that the process of reversion, which should have normally followed in this case within a day or two, should be completely prevented, and that the embryo should go on developing, as it did, *head* *downwards*, and still continue to live. Yet such was the case, and the final result, which I have depicted in Text-fig. 4, is both ludicrous and pathetic. The mature embryo emerged as a pronymph "tail-foremost," until it finally became caught in the egg-shell, and died while still only partially free!



Text-fig. 4.*

Section 5.—*Rectal Respiration in newly-hatched Zygopterid larvæ.*

In November, 1915, a large number of eggs of a common Zygopterid dragonfly (probably *Austrolestes leda* Selys, but not determined with certainty) were found in the tissues of a reed at National Park. These were kept *in situ* in the reed, in water in a Petri dish. Some of them were parasitised by a small Hymenopterous insect new to science. The majority, however, hatched out about a week after they were taken. No attempt was made to observe the actual process of hatching in this case. The principal object which I had in mind was the examination of the caudal gills and tracheal system of the young larvæ. The eggs were examined several times a day. Consequently, a number of freshly hatched specimens, ranging from a few minutes up to two hours old, and all of them perfectly transparent, came under my observation.

The most striking discovery about these larvæ was that, from the time of hatching onward for half-an-hour or more, *regular rectal respiration was carried on with great vigour.* The action is exactly like that observed in the young larva of *Anax*. The rectal cavity is alternately expanded and contracted, so that water is drawn in and expelled quite regularly. No true *tracheal gills* are to be seen, such as are visible from the first in the rectum of *Anax*; but there are two, slightly opaque, longitudinal folds, placed latero-ventrally, and evidently carrying a thickened

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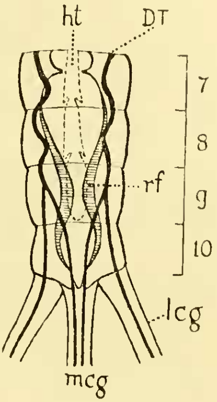
[*] Abnormal hatching of pronymph P* of *Anax papuensis* Burm., "tail-foremost" (×30).

epithelium, which project into the rectum, rising and falling with a somewhat undulating motion. These folds are shown in Text-fig.5. I could not detect any sign of a mid-dorsal fold, such as can be seen in older larvæ. No tracheæ can be seen in these folds.

When the larva is half-an-hour old, or a little older, the regular movements of the rectum cease. Thereafter, the rectum is, for the most part, closed. But, every now and again, it is opened for a short period, during which the movements are again renewed. Larvæ were watched from time to time until they were one or two days old, always with this same result. We must conclude, therefore, that *intermittent rectal respiration* is carried on by young Zygopterid larvæ during the first day or two of their existence, but that it is only *regular and vigorous for a short period directly after hatching.*



Text-fig.5.*

Calvert† has given an excellent summary of the present state of our knowledge of rectal respiration in Zygopterid larvæ. His own experiments on the larvæ of *Calopteryx maculata* (*l.c.*, p.438) are of more value than all the isolated observations of previous authors. By the use of carmine-particles, he proved that this larva expels water regularly from its anal aperture, 33 or more times per minute. He also made similar observations on larvæ of *Hetærina* (*l.c.*, p.440), and *Argia* (*l.c.*, p.442), in both of which he proved that rhythmic pulsations of the rectum were carried on. No observations appear to have been made, up to the present, on newly hatched or very young larvæ.

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* Last four abdominal segments of newly-hatched larva of *Austrolestes leda* Selys; (× 100); dorsal view, to show the paired, latero-ventral, rectal folds(*rf*). DT, dorsal tracheal trunk; *ht*, heart (dotted outline); *lcg*, lateral caudal gill, with single main trachea; *mcg*, median, caudal gill, with two main tracheæ; *rf*, rectal folds; 7-10, abdominal segments.

† "Studies on Costa Rican Odonata. vii. Internal Organs of Larva, and the Respiration and Rectal Tracheation of Zygopterous Larvæ in General." Ent. News, Philadelphia, xxvi., pp.435-447, 1915.

As a general rule, the caudal gills of newly-hatched Zygopterid larvæ are exceedingly slender, almost filiform, and fringed with long, delicate hairs, few in number and irregularly placed. Whatever may be our opinion on the efficacy of these organs at a later period, when they have taken on their final form, and developed within themselves a rich tracheal system, nobody, we suppose, would claim that they are very efficient organs of respiration in the newly-hatched larva. If we combine this well-known fact of the slender and weak formation of these organs with the results of the experiments carried out in Section 2 on the larva of *Anax* (experiments which, one can scarcely doubt, must hold good for the great majority of dragonfly larvæ), we see at once the reason for the vigorous rectal respiration carried on in the case of our Zygopterid larvæ during the first half-hour after hatching. *It is not merely an ordinary act of respiration, but is necessary for the quick replacement of the* CO_2 *in the tracheal system by air.* Once the tracheal system has reached the normal state, so that the necessary supply of oxygen to the tissues is assured, rectal respiration will be governed, in larvæ of different kinds, by *the efficacy of the total of other respiratory activities.* Respiration through caudal gills, through lateral abdominal gills when they occur, and through the integument in such cases as it may occur, all differ from rectal respiration in being *practically continuous*, carried on without special efforts on the part of the larva. Consequently, if the caudal gills have a poor tracheal supply, or a very tough integument, or if the general integument of the larva be unsuitable for respiration by diffusion, the call on the larva for rectal pulsations must be heavier. If the caudal gills are accidentally lost, we should expect that the rate of rectal respiration would be increased. Similarly, we might expect to find a higher rate of rectal respiration towards the end of each instar, when the general integument tends to become tough and hard, than at the beginning, when it is soft and delicate. Experiments along these lines should lead to interesting conclusions as to the true respiratory value of the caudal gills and general integument.

It remains true, in spite of the fact that rectal respiration has now been shown to be carried on both in newly-hatched and in well-grown Zygopterid larvæ, that *no true tracheal gills* are developed in the rectum of these larvæ. The diffusion of CO_2 outwards into the rectal water-supply, and the diffusion of air inwards, is undoubtedly effected through the rectal epithelium *without any specialisation of the tracheal supply of that region*, and the exchange must be regarded as being primarily one between the blood of the larva and the water in the rectum, quite irrespective of the tracheal system, which is bound to benefit sooner or later by the change. Where the Anisoptera differ from the Zygoptera is in the fact that a set of definitely new structures, viz., *tracheal gills*, of great complexity and efficacy, have been developed in the rectum in such a manner as to do away with the need for any other method of respiration, and to bring the necessary oxygen direct from the water to the tracheal system, by means of the thousands of minute capillary loops which lie in the projecting gill-folds. The thickened folds of rectal epithelium in Zygopterid larvæ are structurally not homologous with the gills of Anisopterid larvæ, but rather with their *basal pads*, to which they bear a close morphological resemblance, especially in the more archaic types. It would seem, therefore, highly probable that the *elimination of* CO_2 may be one of the principal functions of these pads, seeing that, before special tracheal gills were developed, such elimination must have proceeded outwards *pari passu* with the entry of air inwards, by diffusion.

In conclusion, we would suggest that there is some definite correlation between the general asthenogenetic trend of development of the Zygoptera as a whole, and their failure to develop any single set of highly efficient respiratory organs in the larval stage. They cannot compete on equal terms with the highly-endowed Anisopterid larvæ in this respect. Inherent weakness in the respiratory system is as fatal to the development of a strong evolutionary line as inherent inability to obtain food easily.

Only in the case of the *Pseudostigmatinæ* does this inability seem to have been removed (not unlikely, it appears, by bringing the stigmata themselves into play for breathing air directly); and the result has been, in this case, the evolution of a race of giants.

SUMMARY.

i. *Conclusions to be drawn from Sections 1-4 concerning Anisopterid larvæ.*

A. *The Gas in the Tracheal System, and the Method of Respiration* (Sections 1 and 2).

1. Before hatching, the tracheal system of the embryo contains no gas, but only a clear pale yellowish liquid, which is the liquid portion of the insect's blood, resembling lymph.

2. During the short pronymphal stage, this liquid is replaced by gas throughout the region coterminous with the midgut; *i.e.*, the gas enters practically simultaneously into the dorsal and ventral trunks and their five pairs of connecting tracheæ in that region. What happens in the visceral trunks could not be observed.

3. By a rapid extension, the gas passes forwards into the head, and backwards into the posterior abdominal segments, until it fills the whole tracheal system, including all the capillaries of the rectal gills.

4. The gas in the tracheæ is almost certainly carbonic acid gas.

5. Regular respiration viâ the rectum sets up a process of diffusion between the capillaries of the rectal gills and the circum-ambient water, so that CO_2 passes out into the water, and a mixture of O and N, closely resembling air, passes into the capillaries, until, finally, the partial pressures of all these gases become the same in the tracheal system as in the water (*i.e.*, N, 610 mm.; O, 150 mm.; CO_2, less than 1 mm.). The experiments suggest that a considerable time must elapse before this final equilibrium is fully established.

B. *The Nature and Action of the Cephalic Heart* (Section 3).

While this organ could not be examined actually *in situ* and in action, the following suggestions are made concerning it, and need to be tested by further experiments if an opportunity offers itself:—

1. The Cephalic Heart is probably not a separate structure, but simply a temporary formation in the œsophagus.

2. It pumps liquid blood (without corpuscles) from the "head-vesicle," *i.e.*, from between the pronymphal sheath and true larval head, viâ the larval mouth, into the œsophagus.

3. The consequent distension of the œsophagus, aided by forward pressure of the midgut, enables the larva, (*a*) to lift the pedicel of the egg, and (*b*) to burst the pronymphal sheath dorsally on the head.

4. The apparent two-chambered condition of this organ is probably due to the rigidity of the tentorial framework, which constricts it while in operation.

C. *General* (Section 4).

1. Deprivation of oxygen for the developing embryo is effectively brought about by removal of the vegetable tissues in which the egg was laid, and restriction of the amount of water and access of fresh air.

2. Of twenty-eight embryos so deprived of oxygen, sixteen died without hatching, six reached the pronymphal stage, and six reached the true larval stage, but died soon afterwards.

3. Eight embryos in eggs confined in small blocks of vegetable tissue, but otherwise subjected to restriction of the amount of water and access of fresh air, in the same way as those in 2, all reached the complete larval stage, and showed little or no signs of impaired health.

4. The single embryo which had not undergone reversion, before being subjected to the treatment described in 1, *continued to develop without reversing*, and finally emerged as a pronymph "tail foremost."

ii. *Conclusions to be drawn from Section 5, concerning Zygopterid larvæ.*

1. Rectal respiration is carried on regularly and vigorously during the first half-hour or so of larval existence.

2. This vigorous respiration is necessary in order to effect a quick exchange of the CO_2 in the tracheal system for air derived from the rectal water-supply.

3. Thereafter, for the first two days of larval existence, rectal respiration is intermittent only.

4. This indicates that, for the rest of larval life, rectal respiration is only *accessory*, and is regulated by the sum-total of efficiency of the other means of respiration used by the larva.

5. No true rectal tracheal gills are developed in young Zygopterid larvæ, but two latero-ventral folds of thickened epithelium are seen to play a prominent part in the rectal movements. No special tracheal development can be seen in these rectal folds.

[Printed off, 8th September, 1916.]